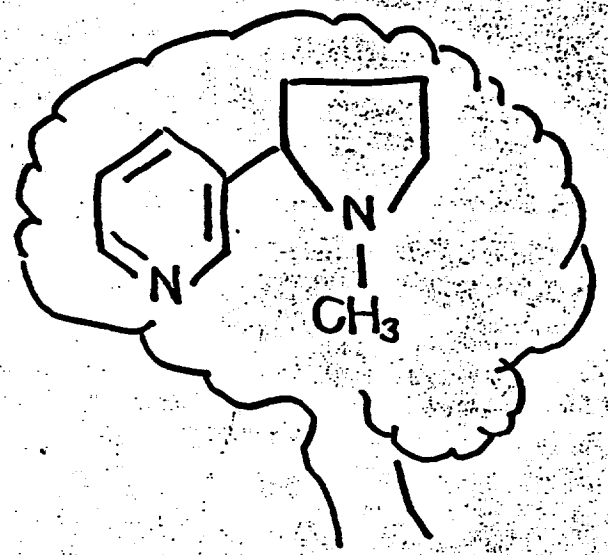
International Symposium on

Nicotinic Receptors in the CNS — Their Role in Synaptic Transmission



ABSTRACTS

Uppsala
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Abstracts

Speakers

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NEUROPHARMACOLOGICAL EFFECTS OF NICOTINE IN RELATION TO CHOLINERGIC MECHANISMS.

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Nicotine and other alkaloids (atropine, muscarine, curare alkaloids) played a key role in the early development of knowledge about the nature and functional organization of the peripheral nervous system. Nicotine in particular was the principal tool used to elucidate the sites of ganglionic transmission in the autonomic innervation of various effector systems. The doses of nicotine used by the pioneers in the study of autonomic physiology and pharmacology were large. Nevertheless, their actions in producing large, albeit transient, increases in blood pressure and cardiac activity which were secondary to activation of nicotinic receptors on autonomic ganglion cells and adrenal medullary chromaffin cells are still used today as evidence for the deleterious effects of smoking on cardiovascular activity, without recognition of the fact that doses of nicotine taken during the act of smoking are orders of magnitude below those producing the dramatic actions that can be demonstrated in animals in reproducing the classical experiments of the early pharmacologists. Studies on the effects of nicotine that are relevant to the amounts taken in during smoking demonstrate that it produces only small cardiovascular effects in those who are non-smokers, and those who are habitual smokers respond to an even smaller extent. There is little evidence that stimulation of autonomic ganglion cells or the adrenal medulla plays a significant part in the effects produced by nicotine self-administered in tobacco smoke. The elucidation of the mechanism responsible for the neuropsychopharmacological effects of nicotine in smoking doses remains to be established: it will certainly not be as straightforward as the elucidation of the classical effects.

Keywords: Nicotine receptors, autonomic ganglia, adrenal medulla, cardiovascular activity.

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PRIMARY CHOLINERGIC, AND INDIRECT DOPAMINERGIC, MEDIATION OF BEHAVIOURAL EFFECTS OF NICOTINE IN RATS.

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Evidence will be presented that behavioural effects of nicotine may be mediated primarily by saturable, high-affinity, nicotinic-cholinergic binding sites for nicotine and secondarily, through dopaminergic mechanisms. Correlations and dissociations between ligand-binding and behavioural data will be presented. The efficacy and potency of certain nicotine analogues in producing the highly specific "nicotine cue" can be predicted from their ability to inhibit binding of [^{3}H]-nicotine. Most non-nicotinic drugs are inactive in both procedures (eg convulsants, anxiolytics, opioids). The main exceptions to the correlation are certain drugs that potently inhibit nicotine binding, but which lack nicotinic-agonist or -antagonist effects. In addition to the direct action at cholinoceptive sites, behavioural effects of nicotine may involve dopamine since certain dopamine agonists partly mimic, and certain antagonists partly block, the nicotine cue. It has been confirmed that microinjections of nicotinic agonists into the ventral tegmental area that contains dopaminergic cells can enhance locomotion (like systemic injections of nicotine), whereas microinjections into several other brain areas have been without effect. These studies suggest that a component in the response to nicotine may be mediated by the mesolimbic dopamine system, perhaps through receptors of the D-1 subtype. The successful future development of this quest for deeper knowledge of brain nicotinic mechanisms will depend on the availability of a wider range of structural analogues of nicotine with differing potencies and profiles of effect.

KEY-WORDS nicotine - nicotinic receptors - dopamine - behaviour - rats

Abstract of presentation at International Symposium on Nicotinic receptors in the CNS - their role in synaptic transmission, Uppsala, June 1988.

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Subjective and behavioural effects of nicotine in humans

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ABSTRACT

Various approaches are described which have been used to study the subjective and behavioral effects of nicotine and smoking in humans. These include (i) Factor analytic studies of self-reported motives for smoking, (ii) experimental studies of the effects of nicotine and smoking on cognitive and motor performance and on mood and other subjective effects, and (iii) studies of the effects of cigarette withdrawal and their alleviation by nicotine. These approaches all show consistency between themselves and with the findings of animal studies and other more fundamental pharmacological studies. It is now well-established that human smoking behaviour is regulated to maintan a fairly constant nicotine intake. Down-regulation to avoid excessive blood nicotine concentrations is very precise, but a drop to lower levels is better tolerated. Some withdrawal effects are alleviated and some performance tasks are enhanced by nicotine at blood levels of about one third of those obtained from smoking.

It is still not known which of the many effects of nicotine are most important in providing satisfaction to human smokers and in the subjective relief of withdrawal. The role of the rate of nicotine absorption and its interaction with acute tolerance (tachyphylaxis) is unclear, but has important implications for the development of better forms of nicotine replacement for the treatment of smokers. Although chronic tolerance to nicotine has been demonstrated in rodents, there is still no systematic study of chronic nicotine tolerance in human smokers and only two studies provide suggestive evidence of a withdrawal 'rebound' after stopping smoking.

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CHOLINERGIC PATHWAYS IN THE BRAIN

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Recent advances in immunohistochemistry and neuroanatomy have led to new insights regarding the organization of central cholinergic neurons. On the basis of this information, cholinergic neurons in the mammalian brain can be divided into 4 major groups: (1) In dopamine-rich regions such as the striatum cholinergic cells are local circuit neurons with very short projections and represent about 1% of the neuronal population. (2) The "cholinergic basal nuclear complex" is delimited by the medial septum rostrally and the nucleus basalis caudally. Computer reconstruction of these neurons has demonstrated that they form a continuum. These neurons have widespread projections to the telencephalon and innervate some diencephalic and mesencephalic structures as well. (3) The "cholinergic mesopontine nuclear complex" is delimited by the caudal substantia nigra rostrally and the laterodorsal tegmental nucleus caudally. These cells also appear to form a continuum. They innervate limited parts of the cerebral cortex and are the major source of the cholinergic innervation of the diencephalon. (4) Somatic motoneurons in the brainstem and spinal cord. The perikarya of the neurons that constitute each of these major groupings are generally large. In addition, in some species scattered populations of smaller cholinergic neurons have been found in other structures including the medial habenular nucleus and the neocortex.

Recent research has focussed on the afferent connections of these cholinergic populations and on the extent to which peptides are colocalized in cholinergic neurons. Galanin is colocalized in some neurons of the cholinergic nuclear basal complex, while substance P, cortocotrophin-releasing factor (CRF) and bombesin/gastrin releasing peptide are colocalized in the cholinergic mesopontine nuclear complex.

Keywords: Cholinergic anatomy, peptide co-localization

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MAPPING OF BRAIN NICOTINIC RECEPTORS BY AUTORADIOGRAPHIC TECHNIQUES AND THE EFFECT OF EXPERIMENTAL LESIONS.

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Biochemical, electrophysiological and behavioural studies point to the existence of central nicotinic receptors in mammalian brain which bear a pharmacological resemblance to ganglionic (C6-type) cholinoceptors. Thus, central receptors are activated by low doses of nicotine in the "smoking" range; nicotinic responses are blocked by C6-selective antagonists, such as mecamylamine, and undergo tachyphylaxis. These receptors appear to be labelled in vitro by nanomolar concentrations of tritiated agonists (nicotine, ACh, methylcarbachol) but not by alpha-bungarotoxin. In the rat brain, the highest density of high-affinity agonist sites occurs in the interpeduncular nucleus (IPN), a structure receiving a massive cholinergic input via the fasciculus retroflexus. Removal of this neural tract reduces the density but not the affinity of ^{3}H-nicotine binding sites within IPN, suggesting that some nicotinic receptors are located on afferent fibres, consistent with previous electrophysiological reports. Lesions employing 6-hydroxydopamine indicate that ^{3}H-nicotine sites are associated with dopaminergic (DA) neurons of the nigrostriatal and mesolimbic systems, not only at the level of cell bodies and/or dendrites, but also in terminal areas. A survey of the evidence suggests that this kind of organisation also occurs in other neuronal systems. Neuroanatomical and electrophysiological experiments suggest that A9 DA neurons receive a cholinergic input which is transduced by nicotinic receptors. Other experiments suggest that autoradiographically-identified receptors on mesolimbic DA neurons mediate the locomotor stimulant actions of nicotine in tolerant rats.

Nicotine - acetylcholine - receptor autoradiography - lesions - electrophysiology - behavioural correlates

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COEXISTENCE OF ACh AND PEPTIDES IN CENTRAL CHOLINERGIC NICOTINIC PATHWAYS T. Melander, T. Hökfelt, G. Fisone

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The occurence of immunoreactive galanin (GAL) and ^{125}I-GAL binding sites in the septum/basal forebrain complex and hippocampal formation was studied with special reference to cholinergic neurons. Effects of GAL on hippocampal cholinergic transmission was analyzed in vitro and in vivo.

Galanin-like immunoreactivity (LI) coexisted with acetylcholine in cell bodies of the diagonal band nuclei and medial septal nucleus projecting to the hippocampus, but apparently not in the neocortically projecting cholinergic system (nucleus basalis) of the rat. In contrast, in the owl monkey (Aotus trivirgatus), a large population of galanin-positive somata was seen also in the different subdivisions of the nucleus basalis in addition to the medial septal and diagonal band nuclei.

Galanin-positive hippocampal fibers were in the rat unevenly distributed and of three types, both with regard to morphology and origin. 1. Coarse, strongly fluorescent fibers originated rostral to the pons, some co-containing serotonin. 2. Fine, faintly fluorescent fibers presumably arrived from locus coeruleus. 3. Punctate fibers originated in the septal complex. In the monkey, galanin-positive hippocampal fibers were more evenly distributed and some co-contained acetylcholinesterase.

With reference to projection areas of the septum/basal forebrain complex, iodinated-galanin binding sites were confined to the ventral hippocampal formation in the rat, whereas in the human and monkey, binding sites were also seen in neocortical areas, corresponded well with the immunohistochemical findings.

Intraventricularly injected GAL (10µg/15µl) inhibited the scopolamine (0.5 mg/kg s.c.) stimulated release of acetylcholine in the ventral but not in the dorsal hippocampus. Lesions of the septo-hippocampal pathway resulted in marked decreases in ^{125}I-GAL binding. The relevance of the results with reference to regulation of cholinergic transmission in the forebrain is discussed.

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MUSCLE AND BRAIN-DERIVED TROPHIC FACTORS INFLUENCING CHOLINERGIC NEURONS IN VITRO AND IN VIVO

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Evidence for the existence of muscle-derived trophic factors derives from studies of the enhancement of survival, neurite elongation, and acetylcholine synthesis and CAT activity of cultured ventral spinal cord neurons. The differential effects or morphological and cholinergic properties are attributable to different protein constituents in muscle extracts. We have recently purified two distinct factors from muscle which stimulate cholinergic activity in vitro. One factor was purified to homogeneity from two-week old rat muscle, and stimulates CAT activity in rat spinal cord neurons. The purified factor is heat-labile, inactivated by proteases, and migrates as a single band on SDS-PAGE and isoelectric focusing with an apparent molecular weight of 20 Kd and a pI of 4.8. It is not retained on heparin-sepharose chromatographic columns. A second factor purified from human muscle tissue is retained on heparin affinity chromatography and is identified as basic FGF. It enhances cholinergic activity in dissociated embryonic chick ciliary ganglion cells.

Basic FGF has been shown to promote survival and neurite outgrowth in cortical and hippocampal neurons. In our laboratory basic FGF not only stimulates neurite outgrowth in septal cultures, but also enhances acetylcholine synthesis and CAT activity. NGF also enhances cholinergic activity in vitro and in vivo. A third constituent which has additive effects on septal cholinergic activity to NGF and FGF has been identified as a central cholinergic trophic factor. Thus both in the periphery and in the CNS diverse agents may exert trophic effects by different mechanisms, at different times, or on different parameters of development.

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MORPHOFUNCTIONAL STUDIES ON THE PROTECTIVE EFFECTS OF NICOTINE ON MECHANICAL AND NEUROTOXIN INDUCED LESIONS OF THE MESO-STRIATAL DOPAMINE SYSTEM IN RODENTS.

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A negative association between smoking and Parkinson's disease has been reported in epidemiological studies.

In the present experiments two types of lesions of the dopamine (DA) system were used, partial hemitransection at the meso-diencephalic junction in male Sprague-Dawley rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced lesions (40 mg/kg, s.c x 2, 16 h time interval) in male C57 black mice. Following the lesions, the effects of chronic treatment with nicotine hydrogen(+)tartrate (0.125 mg/kg/h, Alzet minipumps implanted subcutaneously for two weeks - corresponding to serum nicotine levels of 50.0±5.1 ng/ml) were studied. Semi-automatic image analysis of tyrosine hydroxylase (TH) immunoreactivity (IR) was performed in the striatum and in the substantia nigra. In the unilateral lesion model also ipsilateral rotational behavior after apomorphine (1.0 mg/kg, s.c.) was studied. Furthermore, high performance liquid chromatography (HPLC) of catecholamine stores and quantitative autoradiographic methods to study ^{3}H-nicotine binding and glucose utilization were applied.

The results demonstrated lesion-induced disappearances of TH IR in the striatum and in the substantia nigra after mechanical and MPTP induced lesions. These effects were partly counteracted with nicotine treatment, which also partly counteracted the depletion of dopamine stores in the substantia nigra in the MPTP lesioned mice. The unilateral mechanical lesion induced a marked reduction in ^{3}H-nicotine binding on the lesioned side, as well as a reduction of the striatal glucose utilization. The latter was counteracted by chronic treatment with nicotine. An enhancement of the apomorphine-induced ipsilateral rotational behavior was found in the hemitransected animals after nicotine.

The results indicate an increased survival of lesioned dopamine neurons with chronic nicotine treatment. The protective action may be due to a desensitization of excitatory nicotinic cholinoceptors on the dopamine neurons, which may reduce the firing rate leading to improved ion homeostasis and reduced energy demands.

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ACTIVATION OF CENTRAL PATHWAYS REGULATED BY NICOTINIC RECEPTORS, AS REVEALED BY GLUCOSE UTILIZATION AND CEREBRAL BLOOD FLOW STUDIES

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Smoking appers to have a beneficial effect in Parkinson's disease. Chronic nicotine treatment has recently been found to counteract neuronal impairment in rats with partial hemitransection of the brain stem involving mesostriatal pathways, either by reducing degeneration or by stimulating regeneration. Involvement of metabolic changes and associated alteration in cerebral blood perfusion has been studied by computer-assisted quantitative autoradiography according to Sokoloff's principles. Immediately after hemitransection of Sprague-Dawley rats Alzet minipumps were implanted s.c. to produce a dose of 0.125 mg/kg/hr of nicotine (hydrogen (+) tartrate). Prompt high concentration of nicotine in the brain was secured by four i.p. injections of 0.5 mg/kg nicotine with 30-min intervals just after the operation. Control animals received corresponding volumes of saline throughout. Regional glucose utilization (^{3}H-2-deoxyglucose) and blood flow (^{14}C-iodoantipyrine) were mesured in neighbouring transverse cryostat sections of the brain using a double-isotope technique. Hemitransection significantly reduced the glucose metabolism by a mean of 16.2% compared with the intact side. In nicotine-treated animals the side-to-side difference was eliminated, being even 1.4% higher on the lesioned than the intact side. Similar alterations wee seen in regional blood flow. The mechanism of action has been studied by comparing with effects of the dopamine agonist, EMD 23448 (3 mg/kg twice daily for 2 weeks following operation). In another model the trophic action of nicotine, given according to the above protocol, has been studied with regard to the regenerative capacity of cerebrovascular adrenergic nerves following partial sympathectomy or decentralization of the superior cervical ganglia.

Key words: Nicotine, dopamine agonist, neurotrophic action, partial brain-stem hemitransection, sympathetic nerve lesion, glucose metabolism, cerebral blood flow

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SHORT AND LONG-TERM REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTER PROPERTIES AND BIOSYNTHESIS.

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The acetylcholine nicotinic receptor protein (nAChR) from vertebrate neuromuscular junction and electromotor synapses is an heterologous pentamer ($\alpha_2\beta\gamma\delta$) which contains the ion channel, the binding sites for the neurotransmitter acetylcholine and a variety of "allosteric" sites for pharmacologically active reversible ligands (noncompetitive blockers, Ca^{++}, the thymic polypeptide thymopoietin...) and for phosphorylation. The membrane-bound nAChR undergoes a variety of conformational transitions, in the millisecond to minute timescale, which include the rapid opening of the ion channel and a two-step desensitization toward a high affinity closed state. Such allosteric transitions may serve to model short-term regulation of synapse efficacy at the postsynaptic level by reversible allosteric effectors and covalent modifications.

The evolution of topological distribution and number of nAChR during the formation of the neuromuscular junction involves a complex sequence of processes which include several distinct regulations of gene expression. After an initial enhancement of AChR gene transcription which coïncides with the fusion of myoblasts into myotubes, an activity-dependant repression of AChR biosynthesis takes place in extra-junctional areas. Both can be reproduced in cultured muscle cells and are analysed with probes derived from genomic clones encoding the α-subunit gene. The 5' promoter region of this gene was characterized and shown by transfection experiments to contain sequences important for its tissue specific and developmentally controlled expression.

Calcitonin-gene-related-peptide, a coexisting peptide present in spinal cord motoneurons, increases the number of AChR molecules in chick cultured muscles via intracellular signals distinct from those elicited by electrical activity, pointing to possible differential regulation of receptor genes in junctional vs nonjunctional nuclei.

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EXPRESSION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS FROM cDNA CLONES.

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We have isolated four different cDNA clones which encode four different proteins, each of which is homologous to subunits of the muscle nicotinic acetylcholine receptor. Three of these proteins contain the contiguous cysteine residues that have been shown, in the case of the muscle nicotinic acetylcholine receptor alpha subunit, to react with affinity labeling reagents. We have argued that these three proteins are ligand binding subunits of a family of neuronal nicotinic acetylcholine receptors and have called them the alpha2, alpha3, and alpha4 subunits. The fourth protein lacks these contiguous cysteines and constitutes what we have called the neuronal nicotinic acetylcholine receptor beta2 subunit. We have transcribed RNA from these clones and injected the RNA, in pairwise combinations, into *Xenopus* oocytes. Co-injection of RNA encoding the beta2 subunit with RNA encoding either the alpha2, alpha3, or alpha4 subunits results in the appearance of a functional nicotinic acetylcholine receptor. The receptor is neuronal in that it is not blocked by alpha bungarotoxin. *In situ* hybridization experiments show that the beta2, alpha2, alpha3, and alpha4 genes are widely expressed in the central nervous system. In addition, the beta2 gene is expressed uniquely with either the alpha2, the alpha3, or the alpha4 gene in many parts of the brain. These results show that the nicotinic cholinergic system is widespread in the brain and that there is a diversity of neuronal nicotinic acetylcholine receptors. We suggest that, at least in part, this diversity arises from the combination of a common beta2 subunit with either of three different alpha subunits.

Keywords/phrases - cDNA clones, nicotinic acetylcholine receptor, *Xenopus* oocytes, RNA, *In situ* hybridization, central nervous system, diversity.

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REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR FUNCTION BY PROTEIN PHOSPHORYLATION

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Phosphorylation of the nicotinic acetylcholine receptor in post-synaptic membranes isolated from Torpedo californica is catalyzed by at least three protein kinases, cyclic-AMP dependent protein kinase, protein kinase C and an endogenous tyrosine specific protein kinase. The proposed locations of the seven different phosphorylation sites are all on the major intracellular loop after M3 and adjacent to membrane spanning regions in models of the receptor transmembrane structure, suggesting that phosphorylation might regulate the ion channel of the receptor. The physiological significance of receptor phosphorylation has been examined by measuring the ion transport properties of the receptor following purification, selective phosphorylation by endogenous kinases and reconstitution into liposomes. Reconstituted receptors maintain all the agonist induced cation transport properties observed in vertebrate muscle acetylcholine receptors in situ. Phosphorylation of serine residues on the γ and δ subunits by cyclic-AMP dependent protein kinase increases the rate of the rapid phase of desensitization as measured by quench flow rapid kinetic techniques. Tyrosine specific phosphorylation of the β, γ, and δ subunits by tyrosine kinase also increases the rate of rapid desensitization seen in single channel recordings from isolated patches of liposome-membrane. Phosphorylation, through modifying desensitization behavior, would appear to regulate the efficacy of nicotinic synapses and provide short term modulation of neurotransmission. Reconstituted receptor/ion channel/desensitization/synaptic efficacy/cyclic-AMP dependent protein kinase/protein kinase C/tyrosine kinase

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α-BUNGAROTOXIN BINDING SITES IN THE CNS.

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The α-bungarotoxin binding protein in mammalian brain is a well-characterized biochemical entity. Its biochemical characteristics will be briefly reviewed and compared to those of skeletal muscle nicotinic acetylcholine receptor and the high-affinity agonist binding site in brain.

α-bungarotoxin (BuTX) does not potently block electrophysiological activity in brain, but its receptor is known to possess a binding site for acetylcholine. Data demonstrating that the BuTX receptor is rapidly up-regulated by the administration of nicotine will be presented. These data suggest that the BuTX binding protein interacts *in vivo* with cholinergic agonists. Behavioral studies conducted by us demonstrate that the doses resulting in the up-regulation of the BuTX receptor are probably physiologically relevant and appear to correlate with the alteration of certain behaviors.

Choline also up-regulates the BuTX binding protein, but the time course is different from that of nicotine. Although the up-regulation of the receptor protein by choline is not attributable to a non-specific increase in membrane-bound proteins, it is not likely attributable to the agonist properties of choline. Preliminary data suggest that this effect, however, occurs in the absence of functional pre-synaptic cholinergic elements. When choline is administered in equimolar amounts as either choline chloride or phosphatidylcholine (purified lecithin), the effect on receptor concentration is equivalent.

The results of studies in progress evaluating the effects of neurotoxic lesions of cholinergic terminals in several brain areas on the concentration of BuTX receptors will also be reported.

Keywords: α-bungarotoxin, nicotinic acetylcholine receptors, nicotine, choline

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CHARACTERIZATION OF NICOTINIC RECEPTORS IN AUTONOMIC GANGLIA AND IN THE CNS USING NEURONAL BUNGAROTOXIN

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Neuronal bungarotoxin (NBT), like α-bungarotoxin (BGT), is a peptide neurotoxin isolated from the venom of Bungarus multicinctus. While the two toxins share considerable sequence homology, they differ dramatically in their pharmacological properties. NBT blocks nicotinic receptors in autonomic ganglia, but has little effect on nicotinic receptors in skeletal muscle and electric tissue. BGT has the opposite properties. We have examined the binding of NBT in the chick ciliary ganglion and in cultures of dissociated rat superior cervical ganglion. In both preparations, ^{125}I-NBT binds to two sites, one of which is also recognized by BGT and the other of which is not. Since BGT does not block nicotinic transmission in these ganglia and since it does not prevent the blocking action of NBT, it is the NBT selective site which is most likely the ganglionic nicotinic receptor. Localization of the NBT selective sites by EM autoradiography demonstrates that they are highly localized near synaptic membranes. Estimation of the density of NBT binding sites at synapses are 600 sites/μm^2 in ciliary ganglia and 5000 sites/μm^2 in sympathetic cultures.

We have also found that NBT, but not BGT, blocks the depolarization produced in rat retinal ganglion cells and in intact chick retina by cholinergic agonists. As in autonomic neurons, in chick retina, ^{125}I-NBT binds to two sites, one that is also recognized by BGT and one that is not. The K_d of NBT for the NBT specific site is 2-3 nM and the B_{max} is 100 fmol/ retina. The binding is inhibited by both nicotinic agonists [e.g., nicotine (k_i=400 nM) and dimethylphenylpiperazinium (k_i=700 nM)] and by nicotinic antagonists [e.g., dihydro-β-erythroidine (k_i=1 μM) and d-tubocurarine (k_i=10 μM)]. At the light microscopic level, ^{125}I-NBT binding to the NBT selective site is concentrated in the two laminae of the inner plexiform layer most heavily stained with an antiserum to choline acetyltransferase. These data suggest that NBT is a useful ligand for studying nicotinic receptors in the retina and, perhaps, in other areas of the CNS.

Key words: neuronal bungarotoxin, α-bungarotoxin, BGT 3.1, toxin F, kappa-bungarotoxin, sympathetic, parasympathetic, retina.

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NICOTINIC ACETYLCHOLINE RECEPTOR DIVERSITY: AGONIST BINDING AND FUNCTIONAL POTENCY

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There is yet to emerge a consensus of scientific opinion regarding the relationships between sites (particularly those in the CNS) that interact with putative probes for nicotinic aceylcholine receptors (nAChR) and physiologically functional cholinergic receptors that are targets for the actions of nicotine. In an attempt to address these issues, some of the work in our laboratory has involved studies of the interactions of small cholinergic drugs, curaremimetic neurotoxins, and anti-nAChR antibodies with putative or authentic nAChR from a variety of neuronal and non-neuronal sources. The results of these studies (see Lukas, Proc Natl Acad Sci, USA, 1986) suggested the existence of heterogeneity of toxin binding sites and nAChR-like antigens across and within tissues.

Other studies in our laboratory have concerned the properties of high-affinity binding sites for nicotinic agonists on membrane preparations from Torpedo electroplax, from rat brain, and from two model neuronal cell lines, the TE671 human medulloblastoma of neural tube origin, and the PC12 rat pheochromocytoma of neural crest origin. ^{3}H-labeled acetylcholine (T-ACh) binds to a single class of high-affinity, non-interacting sites (K_D values and Hill coefficients of about 10 nM and 1.0, respectively) on each of the three former preparations. Scatchard plots of T-ACh binding to a small number of PC12 cell sites are best fit by a function that assumes the presence of two classes of sites with macroscopic K_D values of 2 and 40 nM in the ratio of about 1:6. T-ACh binding sites from each of these preparations are distinguishable on the basis of their characteristic sensitivities to blockade by other agonists (such as nicotine, cytisine and suberyldicholine), small antagonists, and neurotoxins. Ion efflux studies also have been used to characterize functional nAChR expressed by the PC12 and TE671 cell lines, and to establish nAChR sensitivity to nicotine and other cholinergic agonists. Each agonist tested exhibits a characertistic dose-response profile for stimulation of nAChR function, but the relative efficacy of a given agonist differs between PC12 and TE671 cells. In the most extreme case, succinylcholine is a potent agonist of TE671 cellular nAChR responses, but is an antagonist when applied to PC12 cellular nAChR. Suberyldicholine and isoarecoline are more potent agonists on TE671 cells, while the selectivity is reversed for nicotine and cytisine, which are more potent agonists of nAChR on PC12 cells. As functional antagonists, curaremimetic neurotoxins are much more potent when acting on TE671 cells, while the 'ganglionic' blocker mecamylamine is selectively antagonistic of nAChR responses on PC12 cells.

The simplest interpretation of these results is that nAChR are a diverse group of macromolecules that express agonist binding subsites with distinctive characteristics. The relationships between high-affinity T-ACh binding sites and sites that regulate nAChR function will be discussed.

PC12 cells, TE671 cells, nicotine, nicotinic receptor, curaremimetic neurotoxin, brain, electroplax, ion efflux

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Heterogenous subtypes of nicotinic receptors in the CNS.

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The agonists ^{3}H-acetylcholine (^{3}H-ACh) and ^{3}H-nicotine have been used to study neuronal nicotinic receptors. Binding studies both to tissue slices and homogenate indicate a colocalization of the high affinity binding in severel brain areas. In addition to the high affinity sites which ^{3}H-nicotine shares with ^{3}H-ACh, ^{3}H-nicotine also labels a low-affinity site. In rat brain ^{3}H-ACh only labels one population of binding sites while two populations have been found in human brain. Also for antagonists, heterogenous binding sites have been found. Thus, have two binding sites been found for ^{3}H-dihydro-beta-erythroidine (^{3}H-DBE) and ^{3}H-tubocurarine (^{3}H-TC), while only one has been found for ^{3}H-bungarotoxin (^{3}H-BTX). It is unclear if the antagonist binding sites share properties with the agonist binding sites. The regional distribution of ^{3}H-BTX binding is different from that of agonist binding, indicating that the ^{3}H-BTX binding sites are distinct from the agonist binding sites.

Ontogenetic studies also indicate the existence of heterogenous subtypes of nicotinic receptors. The number of high-affinity ^{3}H-nicotine binding sites are relatively high in fetus brain. At birth there is a drop in the number of binding sites followed by a continous increase.

Subchronic treatment with nicotine causes an increase in the number of ^{3}H-nicotine high affinity binding sites. This increase can be explained as an interconversion of low-affinity sites to high-affinity sites.

Key words: ^{3}H-nicotine, ^{3}H-acetylcholine, rat brain, heterogenous subtypes, ontogenesis, nicotine treatment.

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STEREOSELECTIVITY OF NICOTINIC RECEPTORS

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In order to identify structural parameters of critical importance for activation of nicotinic receptors, we have determined the conformational preferences of the achiral agonist isoarecolone methiodide [1] and of two potent and stereoselective agonists - (+)-anatoxin a and (-)-cytisine - by use of molecular mechanics and quantum mechanical calculations. Low-energy conformations of these molecules have been used to model a nicotinic pharmacophore and to construct a partial "nicotinic receptor-excluded volume". Our model will be discussed in relation to previously suggested models [2,3]. Using this new model we attempt (a) to predict the stereoselectivity of the new agonist (±)-PHT [4] and (b) to rationalize the stereoselectivity of nicotine on the basis of conformational energetics.

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Keywords: nicotinic agonists, stereoselectivity, receptor mapping, molecular mechanics calculations.

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CHRONIC NICOTINE EXPOSURE AND BRAIN NICOTINIC RECEPTORS-INFLUENCE OF GENETIC FACTORS. Allan C. Collins and Michael J. Marks. School of Pharmacy and Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA.

A series of studies from our laboratory using DBA mice has demonstrated that chronic nicotine infusion results in tolerance to nicotine that is accompanied by increases in the binding of [^{3}H]-nicotine and alpha-[^{125}I]-bungarotoxin (BTX) to several brain regions. Tolerance increases along with the nicotine infusion dose as does nicotine and BTX binding. Tolerance is maximal at an infusion dose of 4 mg/kg/hr and maximal changes in nicotine binding are also seen at this dose. BTX binding continues to increase with further increases in the nicotine infusion dose. A study of the time course of acquisition and loss of tolerance also indicated a close association between the up-regulation and return to control of nicotine binding. Because we have found large strain differences in first dose sensitivity to nicotine and in the development of acute tolerance further studies have characterized the relationship between tolerance development and nicotinic receptor changes in initially two (DBA and C3H) and more recently five (DBA, C3H, C57BL, A, SWR) mouse strains. Dose response comparisons of tolerance development indicated that those mouse strains that are most sensitive to an initial dose of nicotine develop greater tolerance than do those strains that are more resistant. All mouse strains respond to chronic nicotine infusions with a dose-dependent increase in nicotine and BTX binding but more resistant strains are slightly less labile than are sensitive strains. These results support the argument that up-regulation of nicotinic receptors underlies tolerance to nicotine but these results also suggest that genetic factors influence tolerance and receptor changes.

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KEY WORDS: Nicotine . Tolerance Nicotinic Receptors Genetics

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SINGLE CELL STUDIES OF THE ACTIONS OF AGONISTS AND ANTAGONISTS ON NICOTINIC RECEPTORS OF THE CENTRAL NERVOUS SYSTEM.

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The role of nicotinic receptors in central cholinergic transmission has been studied using electrophysiological means. Nicotinic events can be separated from muscarinic events on the basis of time course and susceptibility to nicotinic antagonists. Extracellular electrodes have been used to monitor changes in firing rate caused by microiontophoretic application of acetylcholine to neurons in situ. The predominate effect of ACh measured with this method is an increase in firing rate, and in some areas of the C.N.S., this effect can be ascribed to activation of nicotinic receptors. Intracellular recordings made on in vitro preparations of brain neurons show that the increase in firing rate is caused by activation of a non-specific inward current producing a membrane depolarization. A similar action of ACh in the peripheral nervous system is well substantiated. The central nicotinic effect of ACh is mimicked by nicotine and cytisine, and is blocked by dihydro-β-erythroidine but not by α-bungarotoxin. Thus central nicotinic receptors more closely resemble their counterparts in autonomic ganglia in their sensitivity to different nicotinic antagonists than those of the neuromuscular junction.

Key words: acetylcholine, nicotine, nicotinic receptors, electrophysiology, central nervous system.

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EFFECTS OF NICOTINE ON BRAIN CATECHOLAMINE NEURONS AS STUDIED BY ELECTROPHYSIOLOGICAL TECHNIQUES

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In 1926 Moll reported nicotine to mitigate immobility in Parkinson's disease. Subsequently, Vogt and Kety suggested that brain norepinephrine (NE) and dopamine (DA) systems, respectively, might serve as major neurobiological substrates for nicotine's effect on vigilance as well as its rewarding properties.

Electrophysiological experiments utilizing single cell recording techniques have shown that systemic nicotine administration in small doses causes profound and shortlasting activation of brain NE neurons in the locus coeruleus (LC), an effect which is blocked by administration of both mecamylamine and chlorisondamine. Further evidence suggest that the LC activation by nicotine is at least in part peripherally generated via primary sensory afferents, and relayed to the LC via n. paragigantocellularis. The LC stimulation by nicotine may help to explain ennanced vigilance and coping capacity. Since postactivation inhibition of LC neurons is reduced by nicotine, the impact of e.g. noxious stimuli on LC functioning may be reduced by the drug, allowing continued attentional processes.

Systemic and microiontophoretic administration of nicotine also cause activation of midbrain DA neurons in zona compacta, substantia nigra as well as the adjacent ventral tegmental area (VTA), effects which are antagonized by mecamylamine but not chlorisondamine. Thus, a central site of action involving C_6-nicotinic receptors on the DA neurons is indicated. Our experiments show a specific neuromodulatory action of nicotine on the VTA neurons involving increased bursting activity. This effect probably relates to the euphoriant action of nicotine as well as its effect on motivated behaviour.

Key words: Nicotine, locus coeruleus, mesolimbic, DA neurons, electrophysiology

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REGULATION BY NICOTINE OF MIDBRAIN DOPAMINE NEURONS.

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Studies of nicotinic cholinergic receptors in the mammalian brain have revealed high concentrations of nicotinic high affinity binding sites in both midbrain and target areas of dopamine containing neurons. Previous investigations by our laboratory and others have revealed that nicotine produces multiple excitatory effects on dopamine neurons including behavioral effects as well as enhancing the synthesis and release of dopamine. In the present experiments we have examined the effect of nicotine following its systemic injection, or following injection into target neurons on the firing rate of dopamine neurons in the substantia nigra pars compacta (A9), the ventral tegmental area (VTA; A10) as well as non-dopamine neurons in these regions. For comparison the effects of glutamate were also examined. The changes in the action potential firing frequency and pattern of neurons in the above mentioned regions were recorded by extracellular single units during the i.v. or microinjection of glutamate or nicotine into the respective striatal target regions of the dopaminergic projections: caudate nucleus for A9 and nucleus accumbens for A10.

It was observed that i.v. nicotine in a range of doses between 50 and 200 µg/kg enhanced the activity of A9 cells up to 25% above baseline, irrespective of the preparation. The same range of doses was more than 3 times as effective on A10 DA cells in rats paralyzed and given a local anesthetic. All of the above effects were reversed or prevented by mecamylamine suggesting the involvement of nicotinic-cholinergic receptors. After nicotine-induced stimulation low doses of apomorphine inhibited the firing rate as in control rats.

These results suggest that nicotine shares with other drugs of abuse the characteristic of being more effective in stimulating A10 than A9 neurons. Nicotine injections into the caudate nucleus caused a momentary cessation of the firing rate of SN reticulata neurons but were frequently followed by excitation. The effect of nicotine injections into the caudate on A9 neurons was excitatory. Nicotine injections into the NA caused inhibition of ventral tegmental area non-dopaminergic neurons and excitation of A10 cells. The results suggest that the pharmacological stimulation of the striatum or the nucleus accumbens by glutamate or nicotine influences dopaminergic neurons by removing the tonic inhibitory influence from the adjacent non-dopaminergic neurons.

Key Words: dopamine neurons; substantia nigra; nucleus accumbens; striatum; ventral tegmentum; nicotine; mecamylamine

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INFLUENCE OF NICOTINE ON THE RELEASE OF MONOAMINES IN THE BRAIN.

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This presentation will review the evidence that nicotine alters the secretion of the catecholamines and 5-hydroxytryptamine (5-HT) in the brain. It will deal briefly with changes in noradrenaline secretion and then, in more detail, with the evidence that nicotine stimulates the secretion of dopamine (DA) in the brain. The possible relationship between the increase in the secretion of DA in the mesolimbic system evoked by nicotine and the ability of the drug to act as a psychostimulant and a reward in a self-administration schedule will be discussed. The effects of nicotine on brain 5-HT systems will then be considered. This part of the talk will focus on the evidence that nicotine decreases the concentration and biosynthesis of 5-HT in the hippocampus. New evidence showing that the reductions in hippocampal 5-HT observed in rats treated with nicotine are also found in brain tissue taken at postmortem from human tobacco smokers will be presented. The possible functional and behavioural significance of the changes in hippocampal 5-HT will be discussed with particular reference to their possible association with the expression of nicotine withdrawal effects.

Keywords: Nicotine; Noradrenaline; Dopamine; 5-Hydroxytryptamine; Nicotine Withdrawal.

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EFFECT OF NICOTINE ON THE RELEASE OF ACETYLCHOLINE AND AMINO-ACIDS IN THE BRAIN.

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A number of reports show that nicotine (N) increases acetylcholine (ACh) release in isolated brain preparations (1,2) and in unanaesthetized animals (3). To date, however, little is known about the influence exerted by N on aminoacidergic transmission (4). Thus, the effects N has on ACh, GABA and [^{3}H]-aspartate release were studied in brain slices and in freely moving animals, according to previously described methods (2,3,5).

In vitro. N 18-180μM enhanced the spontaneous and electrically-evoked ^{3}H-ACh release from guinea-pig cortical slices in a concentration dependent manner. This effect was competitively counteracted by D-tubocurarine(2). N also increased both the the overflow of endogenous GABA from unstimulated cortical slices and that of [^{3}H]-aspartate from caudatal slices stimulated at 20 Hz. This facilitatory effect of N was counteracted by D-tubocurarine.

In vivo. The efflux of ACh from the parietal cortex of guinea-pigs and rats provided with epidural cups was increased by N (0.45-0.90 mg/kg i.p. or 1-5 μg i.c.v.) in a dose-dependent manner. The response was less evident in N chronically-treated guinea-pigs. N, at the same doses able to increase ACh release, reduced the tetrodotoxin sensitive GABA efflux. Preliminary experiments show that N increases [^{3}H]-aspartate overflow from the parietal cortex preloaded with this radiolabelling agent.

In conclusion, N affects the aminoacidergic transmission in vivo at the same doses able to stimulate the cholinergic structures. The imbalance between excitatory and inhibitory aminoacidergic signals in the cortex suggests caution in the use of N to increase cholinergic cortical input.

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Key words: acetylcholine, GABA, [^{3}H]-aspartate, nicotine, release, brain slices, freely moving animals.

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PRESYNAPTIC MODULATION OF TRANSMITTER RELEASE BY NICOTINIC RECEPTORS

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An increasing body of evidence from lesioning studies points to the presynaptic localisation in the brain of a significant proportion of nicotinic acetylcholine receptors (nAChR) characterised by high affinity [^{3}H]nicotine binding. We have examined the effect of nicotinic agents on neurotransmitter release from isolated nerve terminals (synaptosomes). In particular, a detailed study of [^{3}H]dopamine release from striatal synaptosomes and [^{3}H]GABA release from hippocampal nerve terminals demonstrates that nicotinic agonists evoke the release of these transmitters in the absence of depolarising stimuli. The half maximal concentration of (-)nicotine is about 5μM in both transmitter systems, and (-)nicotine is equipotent with acetylcholine. Thus smoking doses of nicotine and physiologically relevant concentrations of the endogenous ligand could be expected to stimulate transmitter release. The actions of nicotinic agonists are inhibited by dihydroβerythroidine, mecamylamine and neosurugatoxin, but not by αbungarotoxin. The differential sensitivity to the latter two toxins suggests that the release of neurotransmitter is mediated by nAChR corresponding to high affinity [^{3}H]nicotine binding sites. Nicotine-evoked transmitter release is Ca^{++}-dependent but tetrodotoxin insensitive. We propose that the nAChR can depolarise the nerve terminals directly, via an integral ionophore that opens in response to agonist binding, and this depolarisation triggers transmitter release by the normal Ca^{++}-dependent mechanism.

Keywords: PRESYNAPTIC RECEPTORS, SYNAPTOSOMES, NICOTINE, αBUNGAROTOXIN

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NICOTINE AND OPIOID PEPTIDES

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A number of studies suggest an interaction between nicotine and opioid peptides on several synapses. The present study explores the effect of chronic treatment with nicotine on tissues producing peptides deriving from the opioid peptide precursors proopiomelanocortin (POMC), proenkephalin (PENK) und prodynorphin (PDYN).

High amounts of the POMC-derived peptide β-endorphin are present in the corticotrophic cells of the anterior and the intermediate pituitary. Chronic subcutaneous infusion of nicotine (10 mg/kg/-day) into rats for 7 days causes an increase in the levels of immunoreactive (ir) β-endorphin in the anterior pituitary and a decrease in the levels of the ir peptide in the intermediate pituitary indicating a differential effect of nicotine on the release and/or biosynthesis in the two pituitary lobes.

PDYN-derived peptides (dynorphin$_{1-17}$, dynorphin$_{1-8}$, α-neoendorphin) are synthesized in high amounts by the granule cells of the dentate gyrus of the hippocampus. Chronic treatment of rats with nicotine causes an increase in the mRNA levels coding for prodynorphin and changes in the level or ir dynorphin$_{1-8}$ in the hippocampus. It is possible that the induction in the prodynorphin biosynthesis is caused by the continous activation of nicotinic receptors which are present in a high density on the granule cells of the dentate gyrus.

High amounts of nicotinic receptors are also present on the chromaffin cells of the adrenal medulla. These receptors have been shown to regulate the release of catecholamines and of PENK-derived opioid peptides. Chronic exposure of cultured bovine chromaffin cells with nicotine in vitro causes a marked increase in the level of the mRNA coding for PENK. Since this effect could be blocked by calcium channel antagonists, it appears that the increase in the PENK mRNA levels is due to the influx of calcium ions through voltage-dependent calcium channels which became activated by the nicotine-induced membrane depolarization.

In summary, these results indicate that chronic activation of nicotinic receptors can modify release and/or biosynthesis of each of the three opioid peptide systems in certain tissues.

Nicotine, proopiomelanocortin, proenkephalin, prodynorphin, pituitary, hippocampus, adrenal medulla, mRNA

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ABSTRACT - International Symposium on Nicotinic Receptors in the CNS - Their Role in Synaptic Transmission. 19th-21st June 1988, Uppsala, Sweden

CENTRAL MECHANISMS UNDERLYING THE ACTIONS OF NICOTINE ON NEURO-ENDOCRINE FUNCTION

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The present immunocytochemical studies have demonstrated the existence not only of tubero-infundibular dopaminergic and cholinergic neurons respectively innervating the median eminence but also the existence of a population of arcuate neurons costoring cholinacetylase and tyrosin hydroxylase immunoreactivities, probably representing tubero-infundibular dopamine and acetylcholine costoring neurons. Rapidly desensitizing central nicotinic cholinoceptors are involved in mediating the rapid excitatory effects of nicotine on ACTH, vasopressin, β-endorphin, prolactin and luteinizing hormone secretion. The inhibitory effects of acute intermittent treatment with nicotine on prolactin, LH and TSH serum levels are instead mediated via slowly desensitizing central nicotinic cholinoceptors of the ganglionic type. Increased dopamine release in the median eminence appears to mediate the inhibitory effects of nicotine on LH and prolactin secretion, while increased noradrenaline release within the hypothalamus and the preoptic area may contribute to the excitatory neuroendocrine actions of nicotine. Sex differences in the nicotine induced changes of TSH and ACTH secretion have been demonstrated. Hypothalamic D1 receptors probably regulate the sensitivity of nicotinic cholinoceptors and increase their ability to release hypothalamic noradrenaline. There appear to exist reciprocal interactions between nicotinic cholinoceptors and α2-adrenergic receptors as studied in cortical membrane preparations, opening a new way to control the sensitivity of nicotinic cholinoceptors. Furthermore, a reduced α2-autoreceptor function induced by nicotine may help to explain its ability to increase noradrenaline and adrenaline release. Furthermore, D1 DA receptors in the median eminence in part located on tanycytes partly mediate the inhibitory effects of repeated nicotine treatment on LH secretion, probably related to an unmasking of axonal DA receptors located on LHRH boutons. Thus, nicotine treatment via release of DA onto D1 DA receptors influences tanycytes which form a chemical-physical barrier within the external layer of the median eminence. Finally, perinatal treatment with nicotine has produced persistent increases of DA utilization in the dorsal and lateral palisade zone of the median eminence, which are maintained in adult life and associated with altered neuroendocrine function.

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REGULATION OF BRAIN NICOTINIC CHOLINERGIC RECEPTORS AND ASSOCIATED FUNCTIONS BY NICOTINE.

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The density of nicotinic cholinergic agonist recognition sites in brain is increased by repeated administration of nicotine. One explanation for this increase may be that following its initial stimulatory action, nicotine causes protracted desensitization and eventually functional inactivation of the receptor. Thus, the predominant action of nicotine--its time averaged effect--would be that of an antagonist. To test this hypothesis, the effects of nicotine on prolactin release were measured in rats in which an indwelling jugular vein catheter allowed multiple sampling of blood over time. Nicotine increases plasma prolactin concentrations, which peak 6 to 7.5 min after injection and return to control levels within 30 min. The nicotine-induced increase in plasma prolactin is dose-dependent, completely blocked by pretreatment with mecamylamine (ID_{50} 1mg/kg), and appears to be mediated by receptors in the brain rather than peripherally. As previously reported by Sharp and Beyer (J. Pharmacol. Exp. Ther. 238:1486, 1986), when nicotine is injected a second time, 60 min after the first injection, the prolactin response is very much diminished or completely absent. This is consistent with rapid and protracted desensitization of the nicotinic receptors mediating prolactin release following the first injection. The ED_{50} of nicotine to cause desensitization of the prolactin response to a second injection of nicotine is less than 25 ug/kg. When nicotine is injected chronically for 10 days (0.8 mg/kg, twice daily), the prolactin response to an acute injection of nicotine is absent 2, 4, and 8 days after the last of the chronic injections. This apparent inactivation of nicotinic receptors is present when nicotinic receptor recognition sites are increased by 25-60 percent in most brain areas, including certain nuclei of the hypothalamus. These results are consistent with the hypothesis that the increase in nicotinic cholinergic receptor recognition sites following chronic administration of nicotine derives from the action of nicotine to inactivate the receptors.

Key words: Nicotinic receptor, Brain, Prolactin, Inactivation, Up-regulation

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DIFFERENTIAL BRAIN AND PERIPHERAL NICOTINIC MECHANISMS REGULATING SYMPATHOADRENAL STIMULATION

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In spite of the considerable knowledge of nicotinic regulation of sympathoadrenal catecholamine secretion at sympathetic ganglia and nerve endings and at adrenal chromaffin cells, regulation of central sympathetic outflow, which also affects catecholamine secretion, is poorly understood. We compared initial responses of plasma epinephrine (EPI) and norepinephrine (NE) and development of tolerance to intraarterial (ia) versus intracerebroventricular (icv) administration of nicotine in conscious, adult, male, Sprague-Dawley rats. With ia injection, nicotine produced parallel and almost equal increases in plasma EPI and NE levels, whereas icv nicotine increased plasma EPI predominantly. These data are consistent with stimulation of adrenal medulla and sympathetic ganglia and nerves by systemic nicotine, but activation by icv nicotine of pathways only to adrenal and not to sympathetic nerves. At least a portion of the brain nicotinic receptors which mediate stimulation of adrenomedullary secretion are of the ganglionic type, since icv hexamethonium antagonized the plasma EPI response.

Repeated administration of nicotine either centrally or peripherally resulted in tolerance of the sympathoadrenal responses. However, the patterns of desensitization of brain versus peripheral nicotinic systems differed. Twenty-four hours after icv nicotine, the plasma EPI response to a subsequent icv dose was markedly inhibited, whereas ia nicotine repeated at a similar interval produced identical plasma EPI responses. Tachyphylaxis to systemic nicotine was present when the dosing interval was decreased to 30 minutes.

To determine whether alterations in sympathoadrenal sensitivity to nicotine might affect sympathetic responses to other stimuli, we examined plasma catecholamine responses to restraint stress in rats rendered tolerant to ia or icv nicotine. In general, rats tolerant to systemic nicotine exhibited cross-tolerance to stress, whereas stress-induced increases in plasma EPI and NE were equal in naive and icv nicotine-tolerant rats.

These studies document several differences in the brain versus peripheral regulation of sympathoadrenal catecholamine secretion.

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NICOTINE DEPENDENCE AND TOLERANCE IN MAN: PHARMACOKINETIC AND PHARMACODYNAMIC INVESTIGATIONS

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Pharmacokinetic and pharmacodynamic characteristics of nicotine are important in understanding nicotine dependence. With cigarette smoking, nicotine is delivered rapidly to the lung, arterial blood, and to the brain. The pharmacologic effects of nicotine are experienced rapidly; the intensity of effect appears to be related to both rate of rise and maximal level of nicotine in the brain. Effects of a single dose of nicotine tend to diminish quickly due to distribution of nicotine out of the brain and to the development of tolerance. However, with repeated exposure, as in smokers, nicotine levels accumulate in the body throughout the day. Pharmacokinetic-pharmacodynamic modeling of the heart rate acceleration response indicates a half-life of development and regression of tolerance of 35 minutes in people. The interval at which cigarette smokers smoke cigarettes may be determined by the kinetics of regression of tolerance. Modeling also indicates that tolerance to the cardioacceleratory effect of nicotine is not total. At levels of nicotine achieved by the average smoker, 25% of the effect compared to the nontolerant state persists. As tolerance to actions of nicotine develops through the day, relief of nicotine abstinence symptoms is likely to become an increasingly important reason for smoking.

Keywords: Nicotine, dependence, pharmacokinetics, pharmacodynamics, tolerance

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NICOTINE-INDUCED TOLERANCE AND DEPENDENCE IN RATS AND MICE.

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Research conducted in this laboratory over the last 20 years has focused on mechanisms and sites of nicotine action using in vivo pharmacological procedures. The present investigation evaluated the ability of animals to exhibit tolerance and dependence to chronic nicotine exposure using rat and mouse operant procedures. A pre- and post- paradigm was utilized in which rats and mice were administered chronic nicotine doses either before or after being tested for their ability to maintain food motivated lever-pressing behavior. Prior to the initiation of the chronic nicotine regimen (1.2-1.8 mg/kg, s.c in the mouse; 0.8 mg/kg, s.c. in the rat), the dose-response behavioral disruptive effects of nicotine were established in each experimental/species group. This acute effect curve was shifted to the right in both experimental groups (pre- and post- injection) administered chronic nicotine suggesting that tolerance which developed to the behavioral disruptive effects of nicotine was pharmacological in nature. Thus, just the association of nicotine with the behavioral (pre-nicotine) apparatus was not enough to explain tolerance development. The development of tolerance to post-session administered nicotine was observed in both rat and mouse experiments and we have observed: (1) nicotine-induced tolerance was not antagonized by mecamylamine in the rat; and (2) cross-tolerance to physostigmine has also been observed in the mouse. The data obtained at this time are difficult to interpret, but does support previous research in which we and others suggest that nicotine may have effects on more than one receptor site. In addition to these tolerance studies, we have observed a behavioral withdrawal syndrome in the mouse which appears to correlate with an alteration in hypothalamic beta-endorphin levels.

Key Words: Nicotine, Mice, Rats, Pharmacological Tolerance, Behavioral Withdrawal, beta endorphin.

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BEHAVIORAL AND PHARMACOLOGIC MECHANISMS OF NICOTINE DEPENDENCE IN MAN

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Tobacco self-administration is the behavioral interface between tobacco and tobacco-caused morbidity and mortality. Pharmacologic and behavioral factors operate to produce the compulsive nicotine seeking observed in most users of tobacco products. Nicotine is the primary and critical pharmacologic factor that results in this controlled behavior. Nicotine exposure in man produces direct reinforcing effects, and may also produce feelings of pleasure or satisfaction. The reinforcing effects of nicotine are related to the dose given, as well as to the nicotine delivery system: conventional tobacco products are highly reinforcing and are effective at initiating and maintaining nicotine dependence; nicotine polacrilex gum is a weaker reinforcer and is easier to give up by most people than tobacco, and there is no direct evidence that nicotine gum is effective at establishing nicotine seeking behavior in persons not already dependent upon nicotine. Tolerance to subjective effects of nicotine have been studied in man and recent research confirms that the level of tobacco intake gradually increases over a period of several years before leveling off in most tobacco users. Physical dependence upon nicotine develops with chronic exposure and is marked by a syndrome of withdrawal signs and symptoms that may motivate futher nicotine self-administration. Studies with nicotine polacrilex gum confirm that most signs and symptoms of tobacco withdrawal are specific to nicotine and may be effectively treated by nicotine replacement given via this route; nicotine dose level is critical to efficacy. Recent studies indicate that the effective dose level of nicotine when given via this route is determined by (1) mg nicotine per gum and number of pieces of gum, (2) gum chewing rate, (3) the time that nicotine-containing saliva is held in the mouth, (4) the oral pH which varies when beverages such as coffee and cola are used.

Key words: Nicotine // Dependence // Tolerance // Nicotine Gum // Treatment // Cigarette Smoking // Human // Withdrawal Syndrome

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TREATMENT OF NICOTINE DEPENDENCE IN MAN

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Even though nicotine dependence relatively recently has been acknowledged as a dependence disorder equal to other dependencies with its own diagnostic terms, it has for a long time been pharmacologically treated. In the early history of pharmacological treatment of smoking tranquillizers and stimulants were used to reduce withdrawal and replace the stimulating effects of nicotine.

Another class of common medications used has been anti-cholinergic drugs.

Lobeline and Anabasine, alcaloids with similarities to nicotine, have been used relatively extensively. Alcalizing the urine with sodium bicarbonate, to prolong the circulating time of nicotine, and silver acetate, serving as a mild antabus, altering the perception of tobacco smoke, has also been relatively widely used. Most of these compounds lack well controlled studies. Where trials have been made, effects have either been hard to detect or been very small.

Today early work on corticotrophin with analogues and fluoxetine, a serotonine reuptake-blocker, has started.

Some more work has been done with clonidine, an alpha-2-adrenoceptor agonist that suggests that it could possibly turn out to be an effective tool in treating nicotine dependence.

Nicotine itself has, however, so far been the only drug found to be effective in treating nicotine dependence. It is presently given in several formulations although most of the research has been done with the nicotine gum that is the only form on the market today. The efficacy of nicotine gum will be critically reviewed in terms of its power to alleviate withdrawal symptoms and producing long-term cessation results. Tailoring treatment to the addicts' degree of nicotine dependence will also be discussed.

Tobacco smoking, Nicotine dependence, Pharmacological treatment, Nicotine replacement.

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NICOTINIC RECEPTORS IN THE CNS OF MONKEY AND MAN AS STUDIED BY POSITRON EMISSION TOMOGRAPHY (PET)

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Nicotine is considered to be the primary reinforcer in the use of tobacco. The underlying mechanisms for the effect of nicotine are still relatively unknown but are assumed to mainly be via central nicotinic receptors. In order to study nicotinic receptors "in vivo" N-methyl-^{11}C-nicotine was given i.v. to Rhesus monkeys and humans and the uptake and distribution of ^{11}C-radioactivity in the brain were measured during 60 min using positron emission tomography (PET).

After an i.v. injection of a tracer dose of racemic ^{11}C-nicotine to monkey the ^{11}C-radioactivity in brain peaked within 1-2 min and then rapidly declined with an initial half-life of about 12 min followed by a slower decline. The ^{11}C-radioactivity in blood was lower than in the brain and decreased rapidly with time. Pretreatment with unlabelled nicotine (10 mikrog/kg) decreased the uptake of ^{11}C-radioavtivity with about 30 per cent. Administration of (-)^{11}C-nicotine resulted in different regional distribution of radioactivity while no regional difference was observed following administration of the (+)-isomer. Both experiments were performed during continous infusion of the nicotinic antagonist trimethaphan (0.1 mg/ml/min) to prevent binding of the ^{11}C-nicotine to peripheral nicotinic receptors.

In human brain the ^{11}C-nicotine was readily taken up and distributed to various cortical and subcortical regions. The highest radioactivity was obtained in the frontal and temporal cortex, the anterior cingulum, the insular cortex, the basal ganglia and the thalamus. The lowest activities were in the white matter and in the blood. Peak activity was at 4-6 min in the brain and at 1-2 min in the blood. The half life of the brain activity was about 20 min. There were no differences between the kinetics in blood of (+)- and (-)^{11}C-nicotine. The uptake of (-)^{11}C-nicotine to the brain was higher than that of (+)^{11}C-nicotine, particularly in smokers as compared to nonsmokers.

The results indicate that the PET technique may be used for further understanding of nicotinic mechanisms in tobacco use as well as for investigations of diseases involving central cholinergic transmission such as dementia disorders.

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CHANGES IN NICOTINIC RECEPTORS IN HUMAN AND RAT CNS.

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Among the severe deficits seen in the cholinergic system in Alzheimer's disease (AD), a significant decrease in high affinity nicotinic binding sites has been observed. The effect of cholinominetic therapy with cholinesterase inhibitors or cholinergic agonists may be influenced by presynaptic nicotinic receptor function and acetylcholine (ACh) release. We have examined the effect of long-lasting intracerebroventricular (i.c.v) administration of physostigmine (4 ug, twice daily under 13 days) on ^{3}H-ACh release, nicotinic receptor binding and AChE in various brain regions (cortex, striatum and hippocampus) of the rat. We have also compared nicotinic receptor binding in both bioptic and autoptic samples of human frontal cortex of AD patients and controls. Biochemical findings have been correlated to clinical and pathological diagnoses. Nicotinic receptors were characterized by means of three ligands (nicotine, alpha-bungarotoxin and K-bungarotoxin). Comparable decreases in nicotinic binding were observed in both autoptic and bioptic tissue which were closely correlated to clinical and pathological findings. Our studies suggest that frontal cortex biopsy may constitute a useful complement to clinical and pathological examinations in AD. (Supported in part by National Institute of Aging AG05416).

Key Words: nicotinic receptors, human/rat CNS, Alzheimer

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DIFFERENTIAL ALTERATIONS IN NICOTINIC RECEPTORS IN CORTICAL AND SUB-CORTICAL REGIONS OF HUMAN BRAIN IN ALZHEIMER'S DISEASE

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The main objective of the present study was to determine whether nicotinic receptors are selectively altered in various brain regions in Alzheimer's Disease (AD). Consequently, the affinities (K_d) and maximal densities (B_{max}) of these sites were determined in cortical and sub-cortical areas in AD and age-matched control brain tissues, using [^{3}H]N-methylcarbamylcholine (MCC) as ligand. For comparison, choline acetyltransferase activity (ChAT) was measured in the same brain regions. As expected, ChAT activity was markedly reduced in all cortical areas, hippocampus and nucleus basalis of Meynert (nbm) in AD brains. However, ChAT activity was normal in other sub-cortical structures including the thalamus, striatum and globus pallidus. Similarly, the maximal density of nicotinic sites was markedly decreased in all cortical areas and hippocampus in AD brains, with the most marked alterations found in the temporal cortex and hippocampus. However, nicotinic binding site parameters were not changed in sub-cortical areas including the nbm in AD. The significant correlation observed between alterations in ChAT activity and nicotinic binding sites (labelled by [^{3}H]MCC) in cortical areas suggest that these two cholinergic markers may be associated with presynaptic cholinergic nerve terminals in these brain regions. It remains to be demonstrated if similar alterations in nicotinic receptor sites can also be observed in other brain diseases or are selective to AD. Supported by the Medical Research Council of Canada.

Acetylcholine Nicotine Alzheimer's Disease Autoreceptor Cortex

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THE ROLE OF NICOTINIC RECEPTORS IN THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

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The involvement of cholinergic nicotinic receptors in dementia disorders such as Alzheimer's disease, senile dementia of Alzheimer type (AD/SDAT) is present under investigation. A reduced number of high affinity nicotinic binding sites in cortex of AD/SDAT brains has been observed. A positive correlation between the amount of potassium evoked in vitro acetylcholine (ACh) release and number of nicotinic receptors can be observed in different human cortices. A parallel reduction in ACh release and in number of high affinity nicotinic receptors was found in the frontal cortex of AD/SDAT cortex compared to controls. The loss in number of high affinity nicotinic receptors was parallel to the reduction in choline acetyltransferase (ChAT) activity. This observation might indicate a selective loss in presynaptic high affinity nicotinic receptors. Analysis of changes in nicotinic subtypes showed a change in the proportion of high affinity nicotinic binding sites to low affinity nicotinic binding sites in AD/SDAT cortical tissue. There was also a decrease in receptor affinity especially for the low affinity nicotinic binding sites. A cholinesterase inhibitor tetrahydroaminoacridine (THA) has been reported to improve AD/SDAT patients. In the presence of THA or physostigmine the in vitro release of ACh was enhanched to control level in AD/SDAT brains. The fascilitating effect of THA on ACh evoked release in AD/SDAT cortex might be mediated via nicotinic receptors since the nicotinic antagonists dihydro-β- erythrodine, tubocurarine partially prevent this effect. An interconversion of high affinity to low affinity nicotinic receptors might be in favour.

Key words:
Alzheimer's disease (AD/SDAT), nicotinic receptors, high affinity sites, low affinity sites, receptor subtypes, acetylcholine release, presynaptic receptors.

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PARKINSON'S DISEASE - ITS ETIOLOGY AND SMOKING

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The clinical entity of Parkinson's disease (P.D.) comprises forms of considerable differences in symptomatology and rate of progress. Although heredity has not been demonstrated in twin-studies genetic factors in the etiology have not been ruled out. The existence of MPTP-induced parkinsonism and diet-related (lathurus, satibus and cycad) neurodegeneration has accelerated the search for environmental factors. According to several authors individuals who develop P.D. are about twice as likely to have been non-smokers when compared with controls. It has been suggested that components in cigarette smoke may inhibit the formation or block the action of neurotoxins. However, in some recent epidemiological investigations the inverse correlation between smoking and P.D. has not been confirmed.
Direct pharmacodynamic effects of nicotine such as increased release of dopamine and induction of thyroxine hydroxylase are not evident in the clinic.

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Abstracts

Posters

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NICOTINIC RECEPTORS IN HUMAN BRAIN AS REVEALED BY RECEPTOR AUTORADIOGRAPHY

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Multiple binding sites selective for nicotinic agonists have been identified in human brain using receptor binding techniques. The regional distribution of these nicotinic recognition sites, as measured by nicotinic agonists, varies in different areas of the human brain, with highest binding in the thalamus. High densities of nicotinic binding sites were also found in the thalamus using receptor autoradiographic techniques. In vitro quantitative autoradiography was also used to visualize nicotinic cholinergic binding sites in large cryosections of human brain hemispheres. Similar to membrane receptor binding studies increasing concentrations of nicotinic binding sites were observed in hippocampus < cortex < substantia nigra < putamen < caudate nucleus < thalamus. Quantitative receptor autoradiographic studies using large cryosections of human brain hemispheres allows high resolution anatomical mapping and quantitation of nicotinic recptors in human brain. Moreover, such studies contribute not only to defining cholinergic receptor alterations in neurological disorders as Alzheimer's disease but linking the alterations to known cellular pathology.

Nicotinic, autoradiography, large cryosections, receptors, nicotine, acetylcholine.

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AN IN VITRO BRAIN PREPARATION FOR THE STUDY OF CENTRAL NICOTINIC SYNAPSES.

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Only a few central cholinergic pathways are known; one of them is thought to the optic nerve synapse with optic tectum in amphibia where transmission is operated mainly through nicotinic eceptors (Freeman and Norden 1984).
We have used of the isolated frog optic tectum as an animal model to study the pharmacological properties of central nicotinic transmission.

The in vitro frog optic tectum was continuosly superfused with oxygenated Riger's solution to which known concentrations of the drugs were added. The effects of the tested chemicals were evaluated on tectal monosynaptic field potentials elicited by electrical stimulation of the controlateral optic nerve.

We have previously demonstrated the enhancing property of acetylcholine (ACh) on low amplitude tectal field potentials, a finding consistent with ACh as the putative optic nerve neurotransmitter (Berti and Nistri 1986). In the present study we investigated the effects of nicotinic compounds on tectal synaptic transmission. The agonists nicotine (Nic) (ED50 17 μM) and tetramethylammonium (TMA) (ED50 28 μM) were virtually as potent as potent as ACh plus neostigmine (ED50 20 μM).
The nicotinic antagonist α-bungarotoxin (10-100 μM) depressed the tectal EPSPs and antagonised the effects of exogenously applied ACh without affecting the response to GABA (0.5 mM) or glutamate (1 mM). Rather unexpectedly d-Tubocurarine (Tub) (10 μM) and mecamylamine (Mec) (10 μM) facilitated the synaptic responses while antagonising the ACh effects.
The enhancing property of Mec was not observed in tectal slices in which the only anatomical input to the tectum was represented by the optic nerve. Under these conditions Mec also antagonised the facilitatory effects of ACh and TMA. Mec decreased the excitability of optic nerve terminals suggesting that there are presynaptic nicotinic receptors facilitating transmitter release.
These data support the hypothesis of nicotinic receptors present on both sides pre and post synaptically at the optic nerve-tectal neurone synapse.

Key words : optic tectum; frog; α-bungarotoxin; mecamylamine.

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EFFECT OF NICOTINE ON [^{3}H]-D-ASPARTIC ACID OVERFLOW FROM STRIATAL SLICES.

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The studies concerning the overflow of putative transmitter glutamate (Glu) from brain slices are difficult to perform because 1) a consistent amount of Glu recovered in the perfusate originates from tissue metabolism and 2) a great part of the Glu actually released by the respective neurons undergoes reuptake which cannot as yet be blocked by selective agents.
Here the most suitable conditions for revealing neuronal efflux of [^{3}H]-D-aspartate (Asp) (assumed as marker of glutamatergic cells) and studying the effect of nicotine (N) on it are reported.

Guinea-pig caudatal slices, preloaded with [^{3}H]-D-Asp 3×10^{-7} M for 30 min at 37°C, were superfused as previously described (1). After 1 hour's perfusion the spontaneous tritium efflux was 8.2±1.2 pmol g^{-1} min^{-1}. Efflux increased when the slices were electrically stimulated with alternate pulses of 5 msec duration. The extent of the net extra-release was dependent on $[Ca^{++}]_e$ and was directly related to pulse frequency(5-20 Hz), strength (15-100 mA/cm^2) and number (1200-4800). This electrically-evoked overflow was tetrodotoxin sensitive and could be repeated twice (S_2/S_1 =1) only when the following parameters were selected: bath temperature= 37°C; $[Ca^{++}]$ = 2.5 mM; pulse strength = 30 mA/cm^2; frequency 10-20 Hz; duration of stimulation 2 min. Under these conditions, the net extra release was 9.7±1.4 g^{-1} min^{-1} and the S_2/S_1 ratio was near to 1. N up to 6.2×10^{-4} M did not change the spontaneous efflux of [^{3}H]-D-Asp but increased the electrically evoked overflow when added for 2 min during S_2. N 1.0×10^{-4}M increased the S_2/S_1 ratio from 0.96±0.1 (controls) to 1.51±.23 (P<0.01) and its effect was prevented by D-tubocurarine. Therefore, N enhances stimulus-triggered [^{3}H]-Asp release from striatal slices at the same concentrations increasing acetylcholine release from cortical slices (2).

1) Beani et al., Eur. J. Pharmacol. 44:[illegible], 1977.
2) Beani et al., Naunyn-Schmiedeberg's Arch. Pharmac. 331:293-296, 1985.

Key words: brain slices, [^{3}H]-D-aspartic acid release, nicotine, D-tubocurarine, electrical stimulation.

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Binding of Nicotine Related Alkaloids to Cortical Nicotinic Receptors.

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Nicotinic Binding sites in the brain have been described as being of two major types: a high affinity site (K_H) which is labeled by acetylcholine and nicotine and a low affinity site (K_L) which is labeled by nicotine. It remains unclear which binding site(s) is/are involved in any functional response to nicotine. In an attempt to further characterize these binding sites their affinity for a series of related alkaloids was tested using competition binding studies in homogenates of rat cortex. ^{3}H-ACh, in the presence of atropine, was used to selectively label high affinity sites. Low affinity sites were labeled using a high concentration of ^{3}H(-)-nicotine which labels both high and low affinity sites.

Displacement of ^{3}H-ACh by (-)-nicotine showed a single site of displacement. In contrast, (+)-nicotine, (-)-nornicotine and (+)-nornicotine indicate two sites of displacement (K_{H1} and K_{H2}). K_{H1}, which represented 20-30% of the total specifically displaceable binding, showed higher affinity for the (+)-enantiomeres than the (-)-enantiomeres of nicotine and nornicotine. The major binding site, K_{H2}, differentiated between the (-)- and (+)-enantiomeres of nicotine while the nornicotine enantiomeres were equipotent.

Displacement of ^{3}H(-)-nicotine by nicotine and nornicotine enantiomeres showed the presence of two binding sites, K_H and K_L. The high affinity site displayed the same binding characteristics as K_{H2} found when using ^{3}H-ACh. K_L showed both structural and stereoselectivity. The order of affinity of the enantiomeres at K_L was (-)-nicotine > (+)-nicotine > (-)-nornicotine > (+)-nornicotine.

^{3}H-ACh from the nicotinic binding site. Nicotine iminium, nicotine isomethonium and nicotine N'-oxide showed two sites of displacement, K_{H1} and K_{H2}, while cotinine showed a single site. The order of potency at K_{H1} was nicotine iminium = nicotine isomethonium > nornicotine > nicotine N'-oxide. The order of potency at K_{H2} was nicotine iminium > nornicotine > nicotine isomethonium >>cotinine > nicotine N'-oxide.

This study indicates that the high affinity, nicotinic-acetylcholine binding site may be composed of two binding sites and that (-)-nicotine has a similar affinity for both of these high affinity sites. In addition, the low affinity site displays both stereo and structural selectivity.

Nicotinic receptors High affinity binding Low affinity binding Enantiomeres Nicotine metabolites.

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SPINAL CHOLINERGIC ANALGESIA IN THE RAT: EFFECTS OF NICOTINE AND NICOTINE AGONISTS

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Centrally acting cholinomimetic agents as physostigmine have analgesic effects after systemic administration in man as well as spinally in the rat. The aim of the present investigation was to study the effects of nicotinic agents administrated intrathecally (i.t.) to the rat spinal cord.

Male Sprague-Dawley rats were given doses of nicotinic receptor agonists (nicotine, cytisin, 9-aminotetrahydroacridine (THA)) and the antagonist (mecamylamine) via an i.t. catheter and the effect was evaluated by using the tail immersion test.

After i.t. administration of 40 µg cytisin there was a short lasting and significant increase in the latency time in the test. Nicotine gave none or a very slight increase in latency times as was the effect of THA. In addition, THA gave a decrease in latency time five minutes after administration. The agonists were also associated with side effects as motor disturbances and vocalization. The magnitude of severity of the side effects was in the order nicotine > THA > cytisine. These effects as well as decreased latency times after THA were attenuated or abolished with 15 µg of mecamylamine, indicating a receptor mediated effect. Motor disturbance was pronounced after THA and was not antagonized with mecamylamine.

In conclusion the spinal nicotinic receptor agonists produce only a minor antinociceptive effect in the rat. Due to side effects this principle should be used with care for spinal analgesia. The pattern of side effects are possibly mediated via different types of nicotinic receptors.

Key words: Spinal cord, nicotine, cytisin, 9-aminotetrahydroacridine, analgesia.

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NEURONAL α-BUNGAROTOXIN BINDING SITE STRUCTURE AND MODULATION DURING DIFFERENTIATION

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α-Bungarotoxin (α-Bgtx), a specific marker for muscular nicotinic receptor (AChR) labels several sites in CNS, but their distribution is different from those of ACh and Nicotine, and furthermore this toxin does not block the function of AChR in several parts of the brain. The structure and function of the α-Bgtx binding molecule in CNS is still elusive.

To further elucidate these points, we searched for an in vitro model which selectively expresses this molecule. We have identified a human neuroblastoma cell line, IMR32, in which it is possible to measure a binding of ^{125}I-αBgtx of 200 fmoles/mg protein and very few activated channels (≈2600 αBgtx binding sites versus 1-2 channels/cell).

We have characterized the pharmacology of the αBgtx binding site, its modulation during cell growth and differentiation, and its possible relationship with cellular functions.

We purified by affinity chromatography the α-Bgtx binding molecule and characterized its biochemical and immunological parameters. αBgtx receptor when detergent solubilized and purified sediments at 9.5 S and has an isoelectric point at pH 5.3. When run on SDS gel electrophoresis it gave consistently three major peptides whose molecular weights are: 68, 59 and 51 K. We used monoclonal and polyclonal antibodies to identify the structure of peptides and to compare them with the known subunits of Torpedo or muscle AChR. Antibodies raised against αBgtx binding site have been used for the localization of the αBgtx in the central nervous system.

Key words: α-Bungarotoxin binding site, monoclonal antibodies, human neuroblastoma, K-toxin.

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SELECTIVE ACTIVATION OF LIMBIC DOPAMINE FUNCTION BY NICOTINE

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Theories of drug addiction commonly imply the mesolimbic dopamine (DA) system as an important neuronal substrate for the rewarding and dependence-producing effects of various drugs, while the adjacent nigrostriatal DA system has been implicated in motor control and the pathophysiology of Parkinson's disease. Different types of experimental studies have described various effects of nicotine in different parts of the DA systems.

Here, we describe biochemical results of the acute and repeated administration of nicotine on the terminal areas of these two major DA systems in the rat. Male rats of 200 g were injected with the aromatic amino acid decarboxylase inhibitor NSD-1015 30 minutes before sacrifice, after which the striatum and limbic areas were dissected and homogenized. Tissue catecholamines were determined by high performance liquid chromatography with electrochemical detection.

Acute nicotine (0.2-0.8 mg/kg, s.c.) increased DOPAC levels and DOPA accumulation in limbic, but not in striatal areas. This stimulatory effect on DA function was blocked by pretreatment with the centrally active ganglionic blocker mecamylamine (1.0 mg/kg, s.c.), but not by the quarternary ganglion blocker chlorisondamine (0.1 mg/kg, s.c.). Mecamylamine and chlorisondamine produced no effects of their own on DA parameters.

When nicotine was given in single daily doses of 0.2-0.8 mg/kg for five days, tolerance seemed to develop. A challenge dose of nicotine (0.2-0.8 mg/kg) before sacrifice the sixth day produced no change in the measured DA parameters, and basal synthesis rate and release seemed to be unaffected by the chronic nicotine treatment.

Taken together, our results indicate that, 1) nicotine stimulates mesolimbic, but not nigrostriatal, DA function in moderate doses, 2) the stimulatory effect of nicotine is mediated by central nicotinic receptors of the C6 type, and 3) tolerance seems to develop to the nicotine effect after five days of treatment.

Key words: C6 receptor, dopamine, limbic system, nicotine, striatum, tolerance.

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CHRONIC EFFECTS OF NICOTINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT.

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Local cerebral glucose utilization (LCGU) was measured in 45 regions of the rat brain during chronic nicotine infusion using the quantitative autoradiographic 2-deoxy-D-(1-^{14}C)glucose method described by Sokoloff et al. (J. Neurochem. 28, 897-916, 1977). Osmotic minipumps, filled with l-nicotine, were implanted 14 days before the measurement of LCGU resulting in a plasma nicotine concentration of 77±17 (mean±SEM) ng/ml plasma. LCGU was significantly increased in 6 of the 45 brain structures examined, i. e. globus pallidus, septal nucleus, lateral geniculate body, superior colliculus (superficial grey layer), interpeduncular nucleus and optic chiasm. These results are partly congruent wit previous data of our group obtained during acute nicotine infusion (Grünwald et al, Brain Res. 400, 232-238, 1987) in so far as LCGU was increased in the optic chiasm, the lateral geniculate body, the superior colliculus, and the interpeduncular nucleus. On the other hand, the increased LCGU in the globus pallidus and septal nucleus occured during chronic infusion only. It is concluded that chronic nicotine infusion has distinct effects on the functional activity of several brain structures which are partly identical with those affected during acute nicotine infusion and partly diverging from them.

Key words: 2-deoxyglucose, nicotine receptors, brain function.

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DERMAL ADMINISTRATION OF NICOTINE IN RABBITS.

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The basic knowledge of the distribution and elimination kinetics of nicotine has been revealed through intravenous administration experiments in several species, including man. The purpose of the present study has been to examine the kinetics of nicotine, especially following dermal administration. After getting the basic information regarding kinetic parameters from infusion experiments in the rabbit, the systemic plasma concentrations of nicotine achieved after dermal application of solutions at different pH were compared. Nicotine solutions were dermally applied in plastic chambers (radius 5 mm) attached to the animal's skin, which was gently shaven 24 hours prior to the experiment. Plasma samples were analyzed for nicotine and cotinine by a capillary gas-chromatography method.

Results of the intravenous experiments showed a terminal half-life of the biphasic nicotine concentration versus time curve and a plasma clearance of 1 (± 0.4) hours and 110 (± 10) ml/min/kg (n = 3), respectively. After application of a 10% solution of nicotine base at pH = 10.6 (30 mg, 0.8 cm^2) a steady-state concentration of 50 ng nicotine/ml plasma was reached within 3-4 hours; after removal of the dermal chamber nicotine was eliminated from plasma with a half-life of about 1 hour. The calculated flux rate of nicotine was approximately 1 mg/hour/cm^2.

In contrast to the above findings, a nicotine solution at pH 6.0 was poorly absorbed as judged from near minimal detectable plasma concentrations of nicotine and cotinine. A nicotine influx rate 10-100 times lower than that achieved with the basic solution was calculated. At pH above 10, 99% of the nicotine is present as alkaloid base species, whereas at pH 6.0 the corresponding figure is 1%. The high flux rate found in the present study appears to be dependent upon the non-ionized moiety.

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NICOTINE PREVENTS THE EFFECTS OF RESERPINE ON THE METABOLISM OF DOPAMINE, 5-HYDROXYTRYPTAMINE, AND NORADRENALINE IN MOUSE STRIATUM.

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To investigate the mechanism of action of nicotine its interactions with reserpine were studied on the striatal dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) metabolism. The striatal contents of DA and its metabolites (3-MT, DOPAC, HVA), NA and its metabolite (MOPEG) and 5-HT and its metabolite (5-HIAA) were measured using high performance liquid chromatography. At 20-22 °C in hypothermic mice repeated nicotine administration prevented totally the reserpine-induced decrease of DA content and increase of HVA content. In nicotine treated mice the reserpine-induced increase of DOPAC content and the decrease of 3-MT content were markedly reduced. Neither the reserpine-induced decrease of NA content nor the increase of MOPEG content was found in nicotine treated mice. Reserpine prevents the amine transport system of storage granules and causes depletion of monoamines. Reserpine has been shown to bind to vesicular monoamine transporters in bovine caudate nucleus (1). A common transporter molecule is suggested to be involved in the uptake process of different monoamine storage vesicles (2). We found that repeated nicotine administration was able to inhibit the reserpine-induced changes in striatal DA, NA and 5-HT metabolism. These findings imply that nicotine could inhibit the binding of reserpine to the monoamine transporter of synaptic vesicles. This inhibition could be due to similarities in certain parts of the molecular structure of nicotine and reserpine.

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(2) Scherman, D. (1986). J. Neurochem. 47, 331-339

Keywords : nicotine - reserpine - monoamine metabolism - mouse striatum - monoamine transporters

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PROPERTIES OF PUTATIVE NICOTINIC RECEPTORS IDENTIFIED ON CULTURED CORTICAL NEURONS

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Putative high-affinity nicotinic receptors have been identified on cultured neurons derived from fetal rat brain cortex. The properties of these sites, based on the binding of L-[3H] nicotine to cell membrane preparations or to fixed cells, were found to be similar to those observed in adult brain tissue. Equilibrium binding was saturable, reversible, and stereoselective. Scatchard analyses revealed a single class of sites with a Kd of around 3 nM. The kinetics of nicotine binding were consistent with the conversion of the sites to a high-affinity state. The kinetic rate constants for association and dissociation were 0.014/min/nM and 0.03/min, respectively. The pharmacological specificity of the sites, as judged from competition studies, was the same as that in adult brain. Nicotinic agonists were the most potent competitive inhibitors and antagonists were much less effective. The D-isomer of nicotine was 30 to 40-fold less potent than the L-isomer. Control experiments using cultured cortical glia showed that these cells did not contain high-affinity nicotinic sites, indicating that potential receptor sites are located only on neuronal cells. The results suggest that cortical neurons possess high-affinity nicotine binding sites whose properties are the same as those generally attributed to nicotinic cholinergic receptors in adult mammalian brain tissue. Therefore, this primary culture system appears to be a suitable model for studying the cellular location(s) and function(s) of nicotinic receptors in the brain.

KEYWORDS: Nicotinic receptors, cortex, receptors, nicotine binding, high-affinity receptors, neurons, neuronal receptors

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